TWO EPIPHYTIC ALGAE

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(WITH PLATE XVIII)

Pirulus gemmata, nov. gen.

This minute alga was first found in 1897 growing in a culture with other small algae in the botanical laboratory at the University of Basel, Switzerland. Though the exact source of the culture cannot be stated, it is known to have come from the vicinity of Basel. The alga was next found in a culture taken from some epiphytic mosses and liverworts from Guatemala presented to the writer by Miss F. G. SMITH in January 1909. Though search has often been made for it in different parts of the United States, it has never been observed, and any record of its occurrence in the country has escaped notice by the writer.

In its early vegetative state the alga shows nothing distinctive and might easily be taken for a small *Chlorella* or the germinating zoospore of some higher form (fig. 1), but in the shape of the adult cell and in its mode of reproduction it stands unique among all green algae.

In shape the mature cell is pyriform, either perfectly symmetrical or somewhat irregular, 0.0084–0.0112 mm. in length and 0.0056–0.007 mm. at its greatest breadth (figs. 2, 3). The color is a very light green. The parietal chloroplast is cup-shaped, with the large opening either at one side or at the smaller end of the cell, so that a portion of the cell is always colorless. A large vacuole is present (fig. 5*a*). No starch was found in either the material from Switzerland or in that from Guatemala. In the former no reaction for cellulose was obtained with the chloriodide of zinc, but in the latter a distinct reaction was shown with that reagent. A further difference between the two forms was noted in the number of nuclei. In the Swiss material the mature cells when stained with hematoxylin showed four distinct nuclei, and in some instances eight were counted; while in the material from Guatemala a cell

showed but a single nucleus (fig. 5*b*). In all other respects, however, in the shape and the size of the cells, the nature of the chloroplasts, and mode of reproduction, but slight variations in the two forms occurred. The different results obtained in the membrane tests might possibly be explained by a difference in the reagents used, in which case the only real difference would lie in the number of the nuclei. As this is an internal difference rather than an external one, the two algae have been regarded as forms of the same species.

The most distinctive characteristic of this alga is its reproduction. Whereas all other similar forms multiply either by fission or internal division, producing either zoospores or non-motile gonidia, this form reproduces by budding. The smaller end of the pear-shaped cell elongates, then a slight constriction occurs near the end, and a membrane is put across, forming a cell which is nearly spherical, with a diameter much smaller than the original cell (figs. 3, 4). This new cell does not become detached immediately, but again the smaller end of the parent cell elongates and a second cell is produced between the original cell and the one last formed. This is repeated continuously, and often a chain of 12–14 cells is produced before the cells separate, the oldest of the series being farthest away from the original cell (fig. 6). Growth usually begins in each new cell as soon as formed, so that there is a slight increase in size from the youngest to the oldest of the chain, and often the largest ones of these begin to reproduce themselves before the breaking up of the filament takes place (figs. 7, 8). Instances were observed where new cells were seen to originate from opposite ends of the parent cell, but this was a rare occurrence.

The alga was cultivated in pure cultures under various conditions and in various concentrations of Knop's solution, but was found to vary little except in rapidity of growth and length of filament or chain formed. The greatest increase in the amount of material occurred in 0.2–0.5 per cent Knop's solution and on agar-agar plus 0.2 per cent of the same solution; while the longest chains of cells occurred in the weaker concentrations, such as 0.05 and 0.1 per cent, and on agar with a 0.2 per cent solution. With an increase in concentration there was a regular decrease in the

length of the chain of cells formed, until in 1 per cent rarely more than two cells were found united and growth was very meager. If cultures were old, whatever the concentration of the solution there was almost a complete separation of the cells, except once in a 0.4 per cent Knop's solution, where the plant floated on the surface of the liquid, long chains of cells were found. Even in the longest chain, however, the connection between the cells seemed to be a frail one, the cells becoming dissociated so easily that it is a question whether the alga should be called a unicellular one or a filamentous one. In this respect it resembles closely *Stichococcus*, which is classified by CHODAT (1) as a filamentous form, but which probably should be regarded as a unicellular alga.

In what condition this alga existed in its natural surroundings it is impossible to say, since it could not be observed on account of its minute size; but when first noted in culture, the scattered chains consisted of but two or three cells, and indicated that when it was put in the culture it was in the unicellular form.

The direct cause of this fragmentation of the chains or filaments was not determined, but the process resembled so closely a similar fragmentation observed by KLEBS (3) in *Hormidium nitens* that the cause is probably the same. KLEBS observed in his cultures of *Hormidium* that the fragmentation took place either in an insufficient amount of nutritive salts, or in absence of sufficient moisture, and offers a hypothetical explanation of the phenomenon. His theory is that under these conditions the alga at first ceases to divide and then ceases to grow, but that nutrition may continue for some time. As a result, the cell becomes filled with organic nutritive material, whereby the walls become distended and the layer of cuticula binding the cells together becomes broken and the cells fall apart. LIVINGSTON (4), however, in his work on *Stigeoclonium*, believed that a fragmentation occurred as a result of osmosis, and found that the higher the osmotic strength of the nutritive solution the more readily the dissociation of the cells took place.

The phenomenon of filaments falling apart into individual cells each of which continues to live, and under favorable condi-

tions to grow out into a long filament again, is characteristic of most epiphytic filamentous algae, such as *Stichococcus*, *Hormidium*, *Stigeoclonium*, and the following form, and in all cases, whether there is a specialized mode of reproduction or not, this phenomenon must be most instrumental in disseminating the plant. If in the dry epiphytic condition the plant exists in the unicellular state, as would seem to be the case, the cells must be easily blown from one locality to another, and so bring about a wide dissemination of the species. But during a rainy season, undoubtedly those single cells produce filaments which again become broken up into individual cells when the dry conditions return.

There is but little doubt that the alternating wet and dry conditions of the environment in which they live are the means by which the polymorphic conditions of these aerial forms are brought about. As they change their form according to conditions, assuming the nature of either a filamentous or a unicellular alga, they must be regarded as transitional forms between the unicellular and the multicellular genera, and as such they form a most interesting group.

Pirulus, nov. gen.—Alga unicellular, or forming short, fragile, beaded filaments. Mature cell pear-shaped, color a light green: chloroplast cup-shaped, with large opening at one side or at the smaller end; no pyrenoid present. Reproduction by budding, in which the smaller end elongates and is cut off by a membrane, after which a separation may occur or not.

Pirulus gemmata, nov. sp.—Mature cell 0.0084–0.0112 mm. long and 0.0056–0.007 mm. at broadest portion. Membrane of cellulose; a large vacuole at the center and a nucleus present (4 or 8 nuclei in European form). Often forming fragile filaments of 12–15 cells.

Found on epiphytic liverworts and mosses from Guatemala, also in Switzerland.

Aeronema polymorpha, nov. gen.

Growing constantly on mosses and liverworts in certain places, and occasionally on the surface of flower pots in plant houses, is a microscopic polymorphic alga, which, according to conditions,

may assume the characteristics of a typical unicellular alga, or may take on the nature of a well defined branched filament.

An alga which undoubtedly belongs in this same genus was found first in Basel, Switzerland, and its life history traced under the guidance of Professor KLEBS (5), but the species here given has been found in various places in the United States and under different conditions, but principally in the vicinity of Northampton, Massachusetts. In which phase of its polymorphism this alga existed in its natural habitat cannot with certainty be stated; its size is so minute, that its identity can be determined only in culture, and there the form that it assumes depends entirely on the cultural conditions.

In its filamentous state it resembles most closely *Conferva* and *Bumilleria* in that each cell contains several chloroplasts which have a peculiar light and transparent color, without starch and pyrenoid (figs. 10–14). The profuse branching of this form, however, would prevent its being classified with either of these genera, which are strictly unbranched. In its unicellular condition (fig. 15) it is doubtful if one could distinguish it from a small *Botrydiopsis*. The cells are spherical, with a number of light-colored, parietal chloroplasts, but the fact that it may assume a filamentous state would probably place it far from this form in the generally accepted system of classification.

In the filamentous state so profuse are its branches that it often forms relatively large, more or less spherical complexes in which the branches radiate from a common center with apical growth. In a vigorously growing culture (as in 0.03 per cent Knop's solution) the filaments are cylindrical, and in each cell there are relatively few irregular parietal chloroplasts (figs. 11, 12). Only a single nucleus is present, which sometimes may be seen between the chloroplasts without staining.

It has been found that the concentration of the culture medium exerts a great influence on the form the plant assumes. In a series of cultures made in different concentrations of Knop's solution ranging from 0.05 per cent up to 1 per cent, it was found that from 0.05 to 0.3 per cent there was a regular increase in individuals, also in the length and number of filaments, which

varied from 0.0028 to 0.006 mm. in diameter; while from 0.3 to 1 per cent reproduction decreased, and the length and number of filaments diminished. This reduction in length was accompanied also by a great increase in the diameter of the cells and the assumption of a spherical shape, followed by an almost complete fragmentation of the filaments previously formed (fig. 15). The diameter of these cells reached as much as 0.0228–0.028 mm. In this condition a well defined reaction for cellulose was obtained in the membrane with the chloriodide of zinc, but this was not obtained in the filamentous stage. In some cases a strand of protoplasm was seen to extend from each chloroplast toward the center; this probably extended to the nucleus, though it could not be determined definitely.

The alga reproduces by means of zoospores 0.005–0.0065 mm. long and 0.0018–0.003 mm. broad (fig. 18). They are pointed at both ends, have a single chloroplast, a single cilium, and a pigment spot. They are very amoeboid in their motion, bending back and forth, and changing their shape from time to time. After a period of activity they come to rest, assume a spherical form, and germinate immediately. The zoospores are formed by the successive division of the contents of a mother cell into 2, then into 4, 8, 16, or many segments (fig. 17). Usually there are 16 or 32 from each cell. They may be formed from any cell, but they seem to be formed first in the central cells of a complex rather than in the ends of the filaments. They are liberated by the gelatinification of the whole membrane and its expansion by the motion of the zoospores until one by one the zoospores become liberated and swim away. Often the process is very slow.

In respect to the zoospores, as well as to the vegetative structure, the resemblance to *Conferva* and *Botrydiopsis* is manifested, as all three have amoeboid zoospores with a single cilium; a difference, however, lies in the fact that these zoospores have only one chloroplast, while the others have two.

The mode of germination of these zoospores and the development into filaments seem to be the same as occurs in the genus *Stigeoclonium*. Soon after a spore comes to rest (fig. 19), it divides into two, and then into a chain of four, the two outer

cells developing in opposite directions into filaments, while the two central cells protrude laterally and give rise to two more filaments (figs. 10–12). Any cell of any of these filaments seems capable of producing a branch, and by the continued growth and branching the large complexes are formed, radiating from the two original cells at the center. Early in their history, however, division occurs in these cells in all three directions of space, so that a parenchymatous mass is formed, from which the branches radiate (figs. 13, 14). CIENKOWSKI (**2**), in writing of *Stigeoclonium*, refers to a similar structure from which the branches radiate, and calls it a *sole*.

As the age of a culture increases, especially in cultures of high concentration, the tendency toward the formation of these parenchymatous masses increases until we find large solid structures with but little resemblance to a filamentous alga (fig. 16).

It has long been known that in the Conjugatae certain forms of desmids resemble closely a single cell of corresponding filamentous forms, as, for example, *Spirotaenia* and *Spirogyra; Cylindrocystis* and *Zygnema; Mesotaenium* and *Mougeotia*. A like similarity must be taken into account in the case of *Conferva* and *Botrydiopsis*, as the nature of the individual cells and the reproduction are almost identical. In this new genus it would seem that we have a transitional form between the well defined filament of *Conferva* on one side and the unicellular *Botrydiopsis* on the other. Or possibly *Conferva* might be an intermediate form between this and *Botrydiopsis*, as certainly the branched nature would indicate a higher development than the single filament. But the filament of *Conferva* is less fragile than the filament of this form, so it is difficult to say what would be the most correct classification.

LUTHER (**5**) and WEST (**6**) would place all algae characterized by the distinctive light green shade shown in this form into one class, the HETEROKONTAE, irrespective of their shape, size, filamentous or unicellular nature; but whether one accepts the views of these writers or retains the older classification, the genus *Aeronema* must be placed near to *Conferva* or *Bumilleria*.

Aeronema, nov. gen.—Microscopic branched filamentous alga, filaments according to conditions often becoming beaded and fragmented or forming more or less parenchymatous masses. Chloroplasts several or many in each cell; light in color, without pyrenoid. Reproduction by means of amoeboid zoospores with a single flagellum, a single chloroplast, and a pigment spot.

Found in Switzerland and the United States.

Aeronema polymorpha, nov. sp.—Diameter of filaments 0.0028–0.006 mm. No starch or oil present; membrane (in beaded filaments) of cellulose; 16 or more zoospores formed in a cell, each 0.005–0.0065 mm. long and 0.0018–0.003 mm. broad.

Found in Massachusetts.

The writer wishes here to express her thanks to Miss F. G. Smith for material brought from Guatemala, and to Professor G. Klebs for guidance and inspiration in the original study of these genera.

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EXPLANATION OF PLATE XVIII

All drawings except fig. 16 were made with a Zeiss 3 mm. objective and no. 6 ocular. Fig. 16 was made with a Zeiss 8 mm. objective and no. 6 ocular. An Abbé camera lucida was used in all drawings except in fig. 18, when two of the zoospores were drawn free hand.

Pirulus gemmata, nov. gen.

Fig. 1.—Young cells.

Fig. 2.—Mature cells.

FIG. 3.—Mature cells somewhat irregular because of crowding.

FIG. 4.—Different stages in the formation of filaments.

FIG. 5.—Optical section showing vacuole (*a*) and nucleus (*e*).

FIG. 6.—Mature filaments.

FIGS. 7, 8.—Filaments showing cells reproducing before dissociation.

Aeronema polymorpha, nov. gen.

FIGS. 9–12.—Young filaments.

FIGS. 13, 14.—Older individuals taken from a culture in 0.3 per cent Knop's solution.

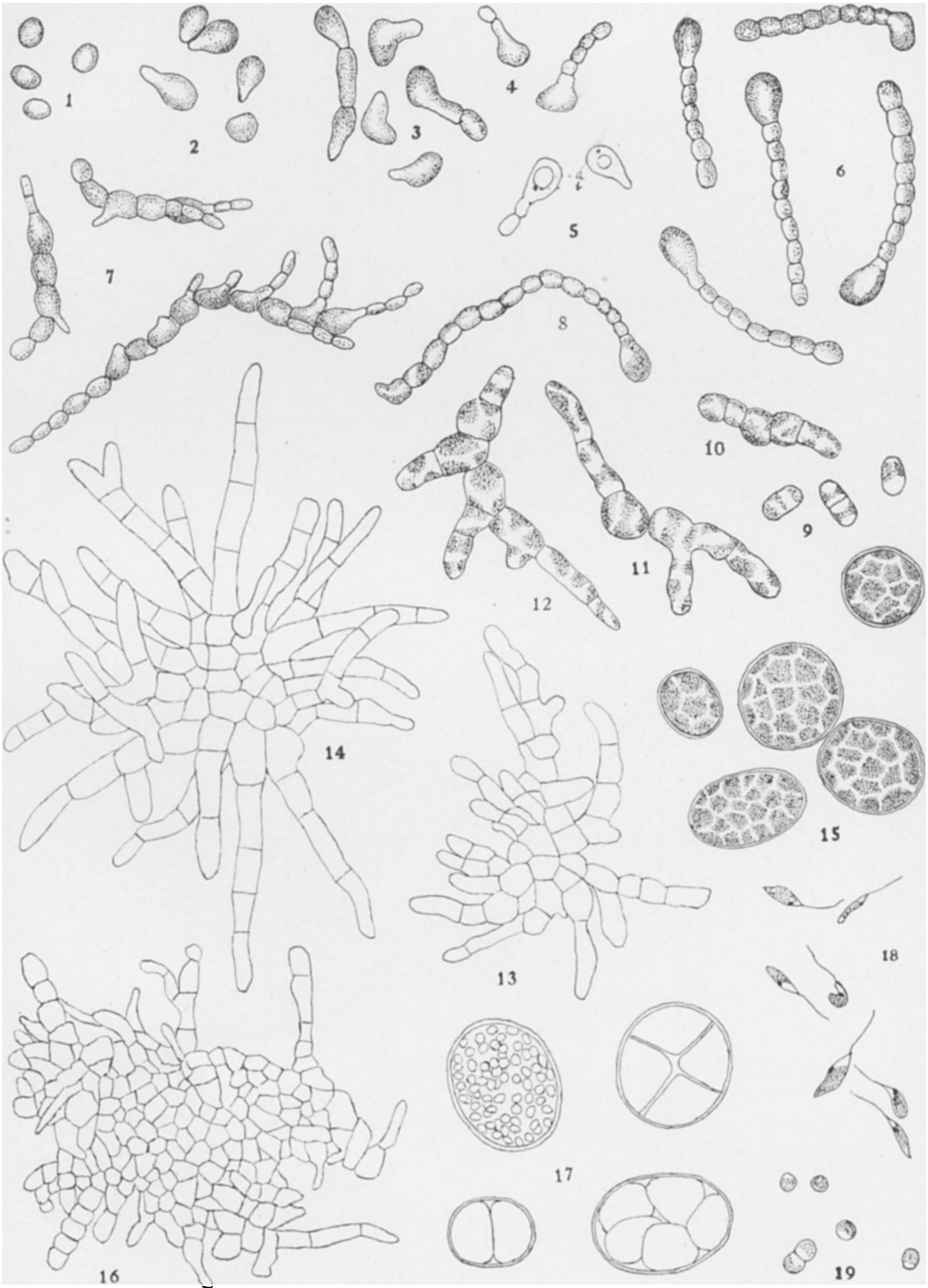
FIG. 15.—Unicellular condition taken from an old culture in 0.3 per cent Knop's solution.

FIG. 16.—Parenchymatous mass taken from an old culture in 0.05 per cent Knop's solution.

FIG. 17.—Different stages in the formation of zoospores from the unicellular condition.

FIG. 18.—Zoospores.

FIG. 19.—Germinating zoospores.



SNOW on EPIPHYTIC ALGAE